Synopsis for Ph. D.

Topic: Bacterial communities associated with fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) and their potential use as pest management

Under the supervision of

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TOPIC

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INTRODUCTION

Tephritid fruit flies (Diptera: Tephritidae) are the most devastating insect pests having a foremost influence on global agricultural products, effecting yield losses, and dropping the value and marketability of horticultural crops**.** The peach fruit fly, *Bactrocera zonata* (Saunders) is one of the most harmful species of Tephritidae. It is polyphagous species attacking more than 50 species of fruit crops and has also been recorded from wild host plants. *Bactrocera zonata* is well adapted in hot climate. This pest is originally an Indian species, firstly reported in Bengal. It is now widespread over India, China, Pakistan, Nepal, Bangladesh, Sri Lanka, Myanmar, Thailand, Egypt and evidently over southeast Asia. *B. zonata* has been recorded on over mainly those with fleshy fruits and flowers like guava, mango, peach, mahua, apple, pineapple included some vegetables etc. Larva infest inside the fruit pulp of insect making it unfit for consumption. It has frequently recovered from peach hence it is called peach fruit fly and also due to its serious damage to guava it is called guava fruit fly (Hussain, 1995).

STATE OF THE ART

The Dipterian family Tephritidae consists of over 4000 species, of which nearly 700 species belonging to Dacine fruit flies (Fletcher, 1987). Nearly 250 species are economic importance and widely distributed in temperate, sub-tropical and tropical regions of the world (Christenson and Foote, 1960). Insects are estimated to harbor endosymbionts including viruses, bacteria, fungi, protozoa, nematodes and multicellular parasites (Buchner 1965; Douglas 1998; Moran *et al.* 2005). The bacterial communities in insects mostly live in the gut, hemocoel, mycetomes and within the cells. The bacteria play a significant role in various metabolic functions of insects, i.e nutrition, digestion, protection, detoxification of insecticides, extreme thermal tolerance and a source of cues and signals synthesis (Dillon and Dillon 2004). They also provide digestive enzymes or vitamins lacking in fruit tissues of their host and improves digestion efficiency which are ultimately manifested in the development, fecundity, and survivability of their hosts (Nakabachi and Ishikawa 1999). Most of the studies related to this pest have been limited to its biology, distributions,

population genetic structure, host plant association and management through pheromone-based mass trapping as well as with chemical insecticides (Duyck et al. 2004; Choudhary et al. 2012, 2018). However, very few studies have been carried out on the bacterial communities associated with *B. zonata*, limited to the culture-dependent techniques (Reddy et al. 2014; Naaz et al. 2016), whereas diversity and functional role of bacterial communities has been investigated in many insect groups including fruit fies (Fitt and O'Brien 1985; Lloyd et al. 1986; Prabhakar et al. 2009; Nakajima et al. 2005; Noman et al. 2019)

PROBLEMS TO BE INVESTIGATED

1. Microbial diversity variation in different developmental stages of pest fruit fly *Bactrocera zonata.*
2. Effect of microbial diversity on insect development.

OBJECTIVES

1. To isolate and characterize the development and sex stages (adult) associated bacterial diversity of peach fruit fly *Bactrocera zonata*.
2. To study the major host-associated bacterial community diversity with peach fruit fly *Bactrocera zonata.*
3. To access the antibiotic sensitivity and attractant efficacy of cultivable bacterial symbionts associated with gut and reproductive organs

MOTIVATION

Types of the bacterial community, behavior, variation in numbers, and types of bacteria in the different development stages of the host could help in the integrated pest management to overcome the pest problem.

HYPOTHESIS

A fruit fly is very important to understand the molecular basis of the interaction of the fruit fly with its host. The molecular characterization of bacterial communities of fruit flies may enable us to plan a well-designed Integrated Pest Management. It may also help us to know the mutuality of host-pest relations.

RESEARCH DESIGN AND METHODOLOGY

COLLECTION AND INITIATION OF THE INSECT CULTURE

1. Fruit fly infested fruits will be collected from the Research Farm of ICAR-RCER, Ranchi and nearby locations.
2. Collection will be done in separate polythene bags
3. Rearing of *B. zonata* will be done for different stages collection and stages separation will be done on the basis of morphological description.

DISSECTION AND ISOLATION OF GUT BACTERIA

1. Different stages of *Bactrocera zonata* will be anesthetized and surface sterilized.
2. Dissected aseptically to remove the gut of different stages and adults' reproductive organs (ovary and testis) separately under laminar airflow.
3. Gut and reproductive tract contents will be streaked on different suitable media (Nutrient Agar, Peptone Yeast Extract Agar, etc.) for bacterial growth at 30±1°C for 48-72 h.
4. A single colony will be separated and streaked onto suitable media (NA, PYEA etc).
5. Predominated bacteria will be isolated through sub-culturing and maintained on respective medium slants or plates at 4 -8 °C

BIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION

1. Morphological, Cultural, Biochemical characterization of the pure culture will be done by standard techniques.
2. Isolates characteristics will be compared with Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 2000).

MOLECULAR CHARACTERIZATION

1. Total genomic DNA of each bacterial isolate will be extracted following the method of Naaz *et al.,* (2016) with some minor modification.
2. PCR amplification of 16S rRNA genes through universal bacterial primers.
3. PCR products will be sequenced and sequences will be identified using online NCBI Blast program.

4. Bacterial isolates sequences will align and phylogenetic tree will be constructed using MEGA ver. software or by using other bioinformatics tools.

ANTIBIOTIC SENSITIVITY AGAINST SYMBIONTS ASSOCIATED WITH GUT AND REPRODUCTIVE ORGANS

1. Maggots will be given the diet with antibiotics such as Trimoxazole, Gentamicin and Norfloxacin.
2. Bacteria with the same stage will be compared control stage through molecular and biochemical method.

SIGNIFICANCE OF STUDY

1. Contributes to the study would provide greater understanding of bacterial communities of stage of *Bactrocera zonata*.
2. Stages, age, and host-associated bacterial diversity-related information will be provided which are important to their survivability and fitness.
3. Information will be used to develop eco-friendly management strategies against this devastating pest.
4. Information will be useful to an understanding of evolutionary knowledge on species establishment.

FUTURE ASPECTS

The future prospect of the present work is to develop comparative studies among fruit fly on economically important fruit plants. Researchers may test the fitness of pests with different types of host plants to calculate the future threat.

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